Remarks

Introduction

Applicant thanks the Examiner for the detailed analysis provided in the Office Action dated December 24, 2004. Claims 4-84, 86-96, 107, 118, 119, 122, 168, 169, 172-176, 189, 194 and 195 are allowed. Claims 3, 171, 190, 191, 193, 196 and 214 are rejected and remain pending. New claims 216 to 223 have been added. While Applicant is aware that the Patent Office usually does not favor the addition of claims after final rejection, Applicant submits that the additional claims are dependant of current pending claims and will not create additional burden to the Examiner.

Applicant notes with appreciation the withdrawal of the objection to claim errors, the obviousness-type double patenting rejection, the outstanding new matter rejection, the outstanding indefiniteness rejections, and the prior art rejections of claim 192 in view of Applicant's amendments of 15 September 2004.

Correction of Claim Errors

Applicant acknowledges the claim errors pointed out by the Examiner. By the foregoing amendments, Applicant has cured the cited typographical claim errors. Specifically, the designation "Previously amended" in claim 193 has been amended to "Currently Amended." In the same claim, the word "an" is now correctly before the word "integration." In claim 214, the misspelled word "wherin" has been deleted. Additional typographical errors, such as extra spacing on line 16 of claim 191, have also been corrected.

Rejections under 35 U.S.C. § 112, second paragraph

On pages 2 – 3 of the Office Action, the Examiner rejected claims 196 and 214 for being indefinite for failing to particularly point our and distinctly claim the subject matter which is regarded as the invention.

Particularly, in claim 196, the recitation of "the coding sequences" lacked antecedent basis in the claim. Further, in claim 214, the phrase "said homologous recombination" also lacked antecedent basis.

In the current amendments, the phrase "the coding sequences" in claim 196 has been deleted and substituted by the phrase "the heterologous DNA sequence." In claim 214, the additions to the claim have provided an antecedent basis for the phrase "said homologous recombination."

Applicant has reviewed the remainder of the claims for errors and indefiniteness and urges that they are free from such deficiencies. Applicant therefore respectfully requests the withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

Rejection under 35 U.S.C. § 112, first paragraph – New Matter

The Examiner rejected the remaining claims for containing NEW MATTER which find no basis in the specification. The Examiner stated that:

> Claims 190-191, 196 and 214 as amended recite NEW MATTER as set forth below. Dependent claims 3 and 171 are also included in the rejection.
>
> Claims 190-191, and 196 recite "control sequences positioned upstream from either of the 5' end or downstream of the 3' end, but not both" (see, e.g., claim 190, lines 4-5). Claims 190-191 and 214 have been amended to indicate that the transcriptionally active intergenic spacer region is not conserved (see, e.g., claim 190, penultimate line). Claim 191 has been amended to recite that a

> promoter is present but another type of 5' control sequence might not be present (see, e.g., lines 5-6). Claim 214 has been amended to recite that no promoter is present but another type of 5' regulatory sequence might be present (see, e.g., lines 4-6). There is no basis on the specification for any of this language or these concepts." Accordingly, the claims are directed to NEW MATTER.

See Office Action, page 3, ¶¶ 3 and 4.

Applicant respectfully disagrees. The use of only one control sequence at one end of a heterologous DNA sequence is disclosed and well-supported by the specification. The Examiner's attention is respectfully drawn to Figure 2A. There, the plasmid map of the pSBL-CG-EG121 vector clearly shows that the Prrn promoter is 5' of the first heterologous DNA sequence – the aadA gene. There is no terminator at the 3' end of the aadA gene. Likewise, there is no promoter immediately upstream of the EPSPS gene – in this example the second and last consecutive heterologous DNA sequence. However, a terminator – the psbA 3' – is situated immediately downstream of the EPSPS gene. The specification is replete with such examples of vectors and expression cassettes which contain more than one gene where each gene is not flanked by both a promoter and a terminator. *See, for example,* Figures 2B, 3A, 3B, and 7B of the Specification.

Moreover, the text of the specification also supports such claims. The Examiner's attention is drawn to page 21, lines 10 – 24, where a preferred embodiment is described. The specification clearly calls for an expression cassette which, in the 5' to 3' orientation, comprises: a promoter, a DNA sequence coding for the desired phenotype, a selectable marker gene, and a terminator. Again, while control sequences flank either sides of heterologous DNA sequences, it is evident from the description that each individual DNA sequence is not flanked by both a promoter and a terminator. Nor is the number of heterologous DNA sequences to be limited to

one: page 25, lines 18 – 20 of the specification discloses that the expression cassette may have "multiple cloning sites for insertion of one or more DNA coding sequences"

To further the prosecution of this application, Applicant has clarified this aspect of his invention in the foregoing amendments to the pending claims and newly added claims. Claim 190 has been amended to again recite the use of 5' and 3' controlling sequences between the one heterologous DNA sequences coding for a peptide of interest. Claim 216, which is dependant upon claim 190, has been added to recite the feature of having at least one additional heterologus DNA sequence coding for another peptide of interest, but where neither coding sequence is flanked by both a 5' and a 3' controlling sequence. Similar changes have been made to claim 196 and claim 219. Applicant therefore respectfully requests that the New Matter rejection be withdrawn.

With respect to claim 214, Applicant has amended the claim to point out that the transcription of the expression cassette is driven by a chloroplast promoter that inherently exists in the transcriptionally active spacer region. As disclosed on page 9 of the specification, the transcriptionally active intergenic spacer regions are "between several genes in the rRNA operon which is transcribed by one promoter. Applicant therefore respectfully urge that the Examiner's rejection is inapposite. Withdrawal of the New Matter rejection is therefore respectfully sought.

Applicant respectfully disagrees with the Examiner's position against the deletion of the "conserved" language in claims 190-191, 196 and 214.

Applicant thus respectfully requests the withdrawal of the NEW MATTER rejection.

Rejection under 35 U.S.C. § 112, first paragraph – Written Description

Claims 3, 171, 190 – 191, 196 and 214 have been rejected under § 112, first paragraph for failing to comply with the written description requirement

Applicant urges that the reasons stated above against the New Matter rejection are equally applicable to the Examiner's written description rejections. The withdrawal of these rejections is therefore respectfully requested.

Provisional Obvious-Type Double-Patenting Rejection over U.S. Patent No. 5,932,479 to Daniell

Applicant appreciates the Examiner's notification that amending the claims to address the NEW MATTER rejection will result in the reinstatement of the judicially created obviousness-type double patenting rejection over U.S. Patent No. 5,932,479 to Daniell. Office Action, page 3, ¶3, lines 2-5.

Applicant by his attorney here addressed and corrected the claims subject to the NEW MATTER rejection. Applicant refers and incorporates the arguments made in the brief entitled "Traversal of the Provisional Obviousness-type Double-Patenting Rejection" filed on 11 September 2003 in the PTO as though stated in full herein. Applicant's current claims, as amended, call for the use of control sequences at either ends of a polycistronic construct comprising at least two heterologous DNA sequences. This is not disclosed or claimed in the prior patent. Moreover, Applicant's 11 September 2003 submission, which is incorporated by reference as though fully stated here, also highlight the differences between Applicant's current claims3, 171, and 190 - 191 and those of his '479 patent. Therefore, Applicant respectfully requests that the Examiner maintain his withdrawal of the obviousness type double-patenting rejection.

Rejection under 35 U.S.C. § 112, first paragraph – Enablement and Written Description

The Examiner has maintained the enablement rejection against the pending claims.

> Claims 3, 171, 190-191, 196 remain, and newly amended claim 214 are rejected under 35 U.S.C. § 112, first paragraph because the specification, while being enabling for claims limited to the intergenic spacer 2 region between the $tRNA^{Ile}$ and $tRNA^{Ala}$ genes of the chloroplast genome of the higher plants, does not reasonably provide enablement for claims broadly drawn to the use of any transcriptionally active spacer region.

See Office Action, page 5, last paragraph. Likewise, the Examiner maintained that the specification is non-enabling for the insertion of heterologous DNA into any transcriptionally active or conserved intergenic spacer region of the chloroplast genome.

> Claims 190-191, 193 and 196 remain, and newly amended claim 214 is rejected under 35 U.S.C. § 112, first paragraph because the specification, while being enabling for claims limited to the intergenic spacer 2 region between the $tRNA^{Ile}$ and $tRNA^{Ala}$ genes of the chloroplast genome of the higher plants for the homologous recombination-mediated insertion of heterologous DNA into the intergenic spacer 2 region of higher plants, does not reasonably provide enablement for the insertion of heterologous DNA into any "transcriptionally active" or "conserved" intergenic spacer region of the chloroplast genome of a multitude of higher plants.

See Office Action, page 6, second paragraph. Moreover, the Examiner has rejected these claims for failing the written description requirement.

> Claims 3, 171, 190-191, 193 and 196 remain, and newly amended claim 214 is rejected under 35 U.S.C. §112, firs paragraph, as failing to comply with the written description requirement. The claims(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

See Office Action, page 9-10, bridge paragraph.

The Enablement Rejection

Ease of Identification of Transcriptionally Active Intergenic spacer regions

Rather than adhering to the prevailing thought of inserting gene expression cassettes into transcriptionally silent spacer regions, the invention teaches the novel concept of inserting expression cassettes into transcriptionally active spacer regions. Particularly, the specification defines a "transcriptionally silent region" as "located between two know divergent promoters of chloroplast genes" where the "promoters transcribe the genes in opposite directions away from the silent region of the chromosome." See Specification, pg. 5-6. Such divergently transcribed, transcriptionally silent regions can be readily identified by those skilled in the art by simply examining the genome maps of different plant species on which the directions of transcription of different genes are usually shown. Thus, any region wherein the transcript is not divergent is suitable for the invention.

Similarly, one skilled in the art could likewise study genome maps to identify transcriptionally active regions. For example, such could be operon regions, or polycistronic transcription units, where several genes are co-transcribed by an upstream promoter as in the exemplified trn^{A}-trn^{I} region. Indeed, the exemplified engine is disclosed as one of sixty such operons or polycistronic transcription units in tobacco in *Sugita et al.* (1996) (of record), pg. 317, Table 2.

In addition, one skilled in the art could also choose a region where the chloroplast genes are each driven by a promoter. Since it is well-known in the art that chloroplast genes do not

have transcription termination units, transcription read-through renders these regions not transcriptionally silent and therefore suitable as insertion sites for this invention.

Many groups have performed chloroplast transformation within these transcriptionally active regions since Applicant's disclosure without difficulty. *Kuroda et al.* (2003) and *Shikinai et al.* (2001) both made use of the *clpP* operon region as a plastid transformation site.[1] *See* Kuroda et al., The Plastid *clpP1* Protease Gene is Essential for Plant Development, Nature (2003) 425: 86-89 and Shikanai et al., The Chloroplast *clpP* Gene, Encoding a Proteolytic Subunit of ATP-Dependant Protease, is Indispensable for Chloroplast Development in Tobacco, Plant Cell. Physiol, 42(3): 264-273 (2001). *Hou et al.* (2002), on the other hand, targeted the *rps7-ndhB* region as an insertion site, where each gene is transcribed by its own promoter with transcription read-through. *See* Hou et al., Chloroplast transformation in oilseed rape, Transgenic Research (2003) 12:111-114. *Mulhauer et al.* (2002) shows insertion into both types of transcriptionally active regions, using the trn^A-trn^I operon-type region as well as the transcription read-through type *ndhF-trnN* region.

The Mühlbauer / Koop group employed chloroplast transformation techniques in their study of plastid DNA replication origins (ORI) in tobacco. *See* Mühlbauer et al. "Funtional analysis of plastid DNA replicaton origins in tobacco by targeted inactivation" The Plant Journal (2002) 32, 175-184. The paper teaches that two ORIs – designated *oriA* and *oriB* – have been found in most organisms and that they are localized at the same sites in tobacco and pea. *Id.* at 175, column 1. In tobacco, *oriA* is localized within the *rRNA* operon and *oriB* downstream from the *trnN* gene. *Id.*, column 2. Both ORIs are found within transcribed regions of the plastid. *Id.* The authors sought to study, through targeted and precise modification of the plastome, the

[1] The *clpP-5'rps12 – rpl20* region is disclosed as a polycistronic transcription unit in *Sugita et al.* (1996) pg. 317, Table 2.

significance of defined sequences for plastid DNA replication in vivo. *Id.* at 176, column 2. Vectors were created to cause the disruption or deletion of the *oriA* or oriB sequences. For investigation of *oriA* located in one of the exons of the *trnI* and *trnA* genes, transformation vectors containing the E.coli *aadA* gene, an artificial ribosome binding site and flanking plastid DNA sequences which allow for homologous recombination and insertion of the foreign gene into the region between *trnI* and *trnA* were constructed. *Id.* For *oriB*, similar vectors were constructed using the flanking plastid DNA sequences appropriate for the region between *ndhF* and *trnN* genes, a tobacco rrn16 promoter, an artificial ribosome binding site, and a 3'UTR element from the *Chlamydomonas reinhardtii rbcL* gene. *Id.* at 178, column 2, figure 3. Plastid transformation was successfully carried out by bombardment. The *aadA* gene, which confers resistance to spectinomycin and streptomycin, was expressed in the resultant transgenic tobacco plants. This paper therefore supports the notion that other transcriptionally active spacer regions exist and those skilled in the art would be able to readily identify them without undue experimentation.

The ease with which other skilled artisans adopt Applicant's teachings to other suitable regions supports Applicant's position that any transcriptionally active region can be used as an insertion site without undue experimentation. Applicant therefore respectfully requests that the lack of enablement rejection be withdrawn.

Applicant has Provided Ample Guidance in the Specification

The specification provides guidance to those skilled in the art on how to identify suitable intergenic spacer sequences. *See* Specification, page 27-28. This method comprises isolating plastid genomic DNA, carrying out hybridization with a radioactive labeled probe of a

known spacer, detecting and isolating plastid sequences which exhibit the desired degree of homology with the probe. Specifically, the specification teaches the use of Southern blots, cleavage by restriction enzymes and hybridization to establish the degree of homology. These techniques were well-known to those skilled in the art at the time of filing of this application. The specification also suggests to those skilled in the art to use known sequences of any intergenic highly conserved spacer sequence as probes to find suitable plastid sequences. *See* Specification, page 27. In view of these teachings, and bearing in mind the relevant community's familiarity with Sugita et. al. (1996) which teaches polycistronic transcription units that are co-transcribed in higher plants, those skilled in the art can identify and use any of the intergenic spacer sequences identified in Sugita as probes for hybridization studies.

As an alternative, the specification also teaches those skilled in the art to use the BLAST program to identify suitable spacer sequences. For ease of reference, description of the BLAST program is attached hereto as Exhibit A.

Those skilled in the art are undoubtedly called upon to engage in some experimentation to locate a region suitable for their purposes. However, enablement is not precluded by some necessity of experimentation. *In re Wands,* 858 F.2d 731, 736-737 (Fed. Cir. 1988); *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1371 (U.S. App. , 1999).("a patent specification complies with the statute even if a "reasonable" amount of routine experimentation is required in order to practice a claimed invention, but that such experimentation must not be "undue."); *Chiron Corp. v. Genentech,* 268 F. Supp. 2d 1148, 1154 (E.D. Ca. 2002) ("enablement is not precluded by the necessity for some experimentation such as routine screening and a patent need not disclose what is well known in the art."). Given the state of the knowledge in the art at the time of the application's filing, and the amount of guidance provided

in the specification, one skilled in the art can readily practice the invention with only a minimum of routine experimentation using commonplace protocol which are used daily in a genetics laboratory. No undue experimentation is called for; therefore, the Examiner should withdraw the enablement rejection under 35 U.S.C. §112, first paragraph.

Applicant's Position Finds Support in the Scientific Community

The Written Description Rejection

To fulfill the written description requirement, the patent specification must clearly allow persons of ordinary skill in the art to recognize that the inventor invented what is claimed. *Chiron Corp. v. Genentech,* 268 F. Supp. 2d at 1161. The guidance provided by the specification, particularly those referred to throughout this submission, certain conveys to one skilled in the art that the Applicant had possession of the claimed subject matter at the time of the application's filing. The specification is replete with examples of using the vectors to transform multiple species of plants. With respect to the use of any transcriptionally active spacer region, the specification has taught that such regions that have been identified are "ubiquitously conserved" and suitable for use with this invention. Indeed, the Applicant discloses that "it is . . . immaterial from which individual spacer of a particular plant the universal vector is constructed." Specification, page 10, lines 29 – 31. As shown above, the Applicant has described the characteristics of intergenic spacer sequences and has provided detailed guidance on how to locate them. Specification, page 27 – 28. It is respectfully submitted that the application fully describes the invention herein.

The above arguments have fully addressed the Examiner's enablement and written description rejections and Applicant urges that the same have been overcome. Applicant respectfully requests that the enablement and written description rejections be withdrawn.

Rejection Under 35 U.S.C. §102(b) – Anticipation by Staub et al. (1995)

Claims 3, 171, 190-191, and 214 have been rejected as anticipated by Staub et al. (1995).

Applicant's arguments which were previously submitted on pages 33-34 of his 15 September 2003 response are incorporated herein as though fully stated. The claims, as amended herein, again call for the use of a universal vector and require integration into a conserved intergenic spacer region. Moreover, unlike the disclosure of the reference which teaches that the uidA coding sequence of interest may lack a 5' promoter, use of a 5' promoter upstream of the first or only heterologous DNA coding sequence is required in all Applicant's claims. Applicant respectfully submits that Staub et al. does not anticipate Applicant's invention. The § 102(b) rejection should therefore be withdrawn.

Conclusion

In so far as the above amendments and remarks have addressed fully the Examiner's rejections in the Final Office Action dated December 24, 2004, the instant application is seen to be in condition for allowance. In view of the foregoing, withdrawal of the Examiner's rejections and issuance of a Notice of Allowance of all pending claims is therefore respectfully requested.

Respectfully submitted,
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Dated: 06/24/04

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